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Reports  
Meyer,  
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LIPIDIUM PAPILLIFERUM POPULATION  
BIOLOGY : HABITAT REQUIREMENTS, LIFE  
HISTORY EXPRESSION, AND SEED BANK  
DYNAMICS



***Lepidium papilliferum* Population Biology:  
Habitat Requirements, Life History Expression, and Seed Bank Dynamics**

Co-Principal Investigators: Susan Meyer, Dana Quinney, and Phil Allen

USDA Forest Service, Shrub Sciences Laboratory, Idaho Army National Guard,  
and Department of Plant Sciences, Brigham Young University

**FINAL REPORT**

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**INTRODUCTION**

This research project was carried out from April 2004 through April 2005. It consisted of four quasi-independent study components designed to address the following questions:

- 1) Given that LEPA is generally confined to slick spot microhabitats, and that the majority of superficially suitable slick spots are not occupied by the species, are there attributes of slick spots that actually support LEPA populations that distinguish them from unoccupied slick spots?
- 2) The best evidence for the continuing presence of LEPA in slick spots where current-year plants are not found is the presence of viable seeds in the seed bank. Does knowledge of the past history of LEPA occupation as well as disturbance regime and soil structure enable us to predict which slick spots will contain viable LEPA seed banks?
- 3) One of the effects of trampling disturbance may be to bury the LEPA seed bank too deeply for successful emergence. To evaluate the viable LEPA seed bank accurately, we need to ask, what is the maximum depth from which LEPA seedlings can successfully emerge?
- 4) Our field demographic studies have demonstrated that LEPA has a dual life history expression, with the majority of plants functioning as summer annuals but with a sometimes significant minority functioning as biennials that flower with the annual cohort of the following year. We performed greenhouse experiments with the seed progeny of known annual and biennial individuals to ask, is the decision to flower vs. remain vegetative the first summer after seedling emergence a plastic response to varying resources, or does it have a genetic basis?

**METHODS**

**Study Question One: LEPA Habitat Requirements**

Close examination of several inhabited LEPA microsites has shown that they have a distinctive three-layer soil structure. The surface layer consists of pale tan silt much like the surface horizons of the surrounding matrix soils, but this layer is generally much thinner than in

the surrounding soils, in which the loess-derived silt layer may extend downward a meter or more. The second layer is a weakly cemented tan argillic horizon that we refer to as the hardpan layer; this layer is also usually quite thin. The third layer consists of bright red blocky clay that extends downward to considerable depth. We have observed that the red clay layer retains moisture much longer into the summer than the silt layer at comparable depths in the surrounding matrix soils. This is probably partly due to the fact that the microsites are areas of water collection, so that there is more water in the subsurface soil at the end of spring. But the effect of the clay hardpan layer as a 'lid' on this soil water reservoir may also be very important. LEPA plants apparently almost invariably penetrate through cracks in the clay hardpan layer and extend their roots into the relatively moisture-rich red clay layer, enabling them to function as summer annuals in this summer-dry environment.

The hypothesis we tested in this study component is that the three-layer structure is the defining characteristic of microsites that are actually occupied by LEPA, because this structure is necessary for the persistence of plant-available water in the subsoil into the summer growing season. We hypothesize that microsites that do not have this structure, either because it never developed or because it was destroyed by trampling disturbance, will be unable to support LEPA.

To test this hypothesis, we characterized a series of microsites with known histories of LEPA occupancy (i.e., a continuum from LEPA never observed through consistently occupied each year by LEPA, based on long term monitoring data) in terms of their soil structure. We sampled from 15 to 25 microsites in each of three populations (Orchard Corner, Red Tie, and Quarry Butte, all on the Orchard Training Area). The microsites in each area were selected to represent the full range of physical and ecological attributes, including size, shape, depth, topographic position, cryptogamic crust cover, litter cover, degree of invasion by exotic winter annuals, evidence of past trampling (irregular surfaces, hoof marks, red clay stains on the surface), and proximity to a known occupied LEPA microsite.

The sampling protocol for each microsite was to extract soil from 10 locations (samples ca. 3 cm x 3 cm in diameter), two from the center, two from the edge, and six from the intermediate zone between the center and the edge (the middle). Each sample location was characterized in terms of the presence and thickness of the surface silt layer, the hardpan layer, and the red clay subsurface layer. In addition, at each slick spot, four cores to the depth of the red clay layer were obtained from soils in the adjacent matrix vegetation, and the thickness of the silt and hardpan layers was determined.

The physical and ecological attributes listed above, as well as indications of the level of current occupancy by LEPA, were recorded for each microsite. These soil samples were also used to evaluate the presence and size of the in situ LEPA seed bank (see below).

For purposes of sampling, microsites were classified into five condition classes based on their history of LEPA occupation and their gross appearance. These classes were:

- 1) Was a slick spot, used to have LEPA, has lost slick spot appearance
- 2) Still looks like a slick spot but disturbed, had LEPA in the past
- 3) Looks like a good slick spot but never had LEPA that we know of
- 4) Good slick spot, supports LEPA now
- 5) Exceptional slick spot, supports lots of LEPA now

At Orchard Corner, five slick spots in each of the five classes were sampled for a total of 25 slick spots. Slick spots in classes 3, 4, and 5 were sampled in June 2004, before shatter of current-year seed. Slick spots in classes 1 and 2 were sampled in September 2004. At Red Tie and Quarry Butte, 5 slick spots in each of classes 3, 4, and 5 were sampled in June 2004 for a total of 15 slick spots in each area. Slick spot samples for classes 1 and 2 in these areas were not obtained and could not be included in the analysis.

### **Study Question Two: LEPA Seed Banks**

Because LEPA is an ephemeral species that does not necessarily recruit actively growing individuals every year, it can be difficult to know for certain at any point in time whether a given microsite actually supports a LEPA population. LEPA relies on a persistent seed bank to provide opportunities for recruitment even following periods of many years with unfavorable conditions. We hypothesized that the presence of viable seeds in the seed bank would be the best indicator of population viability in lieu of presence of above-ground evidence of occupancy. In this study segment, we estimated the size of the LEPA in situ seed bank in each of the microsites.

Seed bank samples were taken by layer from each sample location, and the thickness of the layer was noted. The top 2 cm of the red clay layer (found in every sample) was collected for seed bank analysis. This yielded approximately 1650 seed bank samples (55 microsites x 10 sample locations per microsite x 3 layers per sample location). The goal was to correlate LEPA seed bank attributes with microsite attributes over a large number of microsites rather than to perform a painstakingly accurate sampling of only a few microsites. Our hope was that ten samples would be sufficient to detect the presence of a LEPA seed bank. The seed bank samples were processed as described in Meyer et al 2004. We wet-sieved the samples to remove the soil, then hand-picked apparent LEPA seeds from the non-soil fraction after drying. These were tested in petri dish experiments and scored as germinable intact, germinable after piercing, not germinable but viable by TZ staining, or nonviable.

### **Study Question Three: LEPA Seedling Emergence**

Microsite disturbance does not have to destroy LEPA plants or seeds outright in order to have a deleterious effect on long term population persistence. One possible effect of trampling disturbance is the disruption of the in situ seed bank caused by too-deep burial. In order to evaluate this effect, we need to know how deep is too deep for successful LEPA seedling emergence. This information is also useful in interpreting seed bank data. We approached this question through simple growth chamber experiments. Using slick spot surface silt material from the study area as the planting medium, we sowed LEPA seeds at a series of depths and scored the timing and percentage of subsequent seedling emergence. We performed two experiments. The first experiment had a randomized block design with three blocks and four planting depth treatments (2, 5, 10, and 20 mm) for a total of 12 experimental units. It was carried out at intermediate temperature (10:20C night:day temperature 12h:12h). The second experiment was carried out at two additional temperatures (5:15C and 15:25C) to test the hypothesis that seeds would be able to emerge from greater depth under cool conditions where

respiration would be minimized. Because we obtained substantial emergence from 20 mm in the first experiment, we included an additional depth treatment, 30 mm, in the second experiment. For each experimental unit, 50 LEPA seeds were planted at the assigned depth. The LEPA seeds were imbibed, pierced, and chilled for 4 weeks at 3C to render them germinable prior to planting.

The planting medium was wetted approximately to saturation. The seeds were then placed on the surface of the wet soil, and a covering of damp soil to the correct depth (after light firming) was added. Water was added with a fine mister as necessary during the course of the experiment, which was continued until three days after the last seedling emergence was observed. Seedling emergence data were analyzed using Analysis of Variance appropriate to the randomized block experimental design.

#### **Study Question Four: Life History Expression**

In order to address the question of whether the annual vs. biennial life history expression in LEPA has a genetic basis, we carried out a greenhouse study using seeds of known annual and biennial parents. The null hypothesis was that seedlings of both annual and biennial parents would reach the threshold size for flowering and would flower under greenhouse conditions. This would support the hypothesis that there is no genetic basis for the variation in life history expression, i.e., that it represents a plastic response to resource availability. The alternative hypothesis, that there is a genetic basis for the life history variation, would be supported if the progeny of biennial parents failed to flower under non-limiting greenhouse conditions, while the progeny of annual parents flowered successfully.

Under the hypothesis of control of flowering through resource availability, it is necessary to propose a photoperiod requirement for flowering. Otherwise potentially biennial plants would flower in the field once they reached the threshold size, for example in the fall. Flowering in the field is strictly confined to early summer for both annuals and biennials and is thus very likely under photoperiod control. We tested this hypothesis by growing the plants under increasing day length.

The life history expression experiment was carried out by imbibing, piercing, and chilling seeds prior to planting, as described above. We used a standard potting mix amended with 10% LEPA field soil to create non-limiting conditions for growth. We selected seed progeny of 14 known annual plants and 14 known biennial plants for use in the experiment. For each seed parent, 14 seeds were planted into two blocks, with 7 seeds in each block. The planting containers were Ray Leach conetainers (10 cubic inch volume), which fit in racks that have fourteen rows of seven tubes. Two racks of planted tubes (i.e., seven seeds from each of fourteen annual individuals and seven seeds from each of fourteen biennial individuals for a total of 196 planted cells) comprised a block.

## RESULTS

### Study Question One: LEPA Habitat Requirements

Slick spots representing condition classes four and five, where LEPA was generally present at the time of sampling, were similar to each other and varied little across three geographic areas (Figure 1). The surface silt layer in these occupied slick spots varied from a few millimeters to 3 cm in thickness, with a mean of 2.2 cm, while the hardpan layer varied from 1 to 3 cm in thickness, with a mean of 2 cm. The red clay layer was consistently found below the hardpan layer; its thickness was not determined quantitatively.

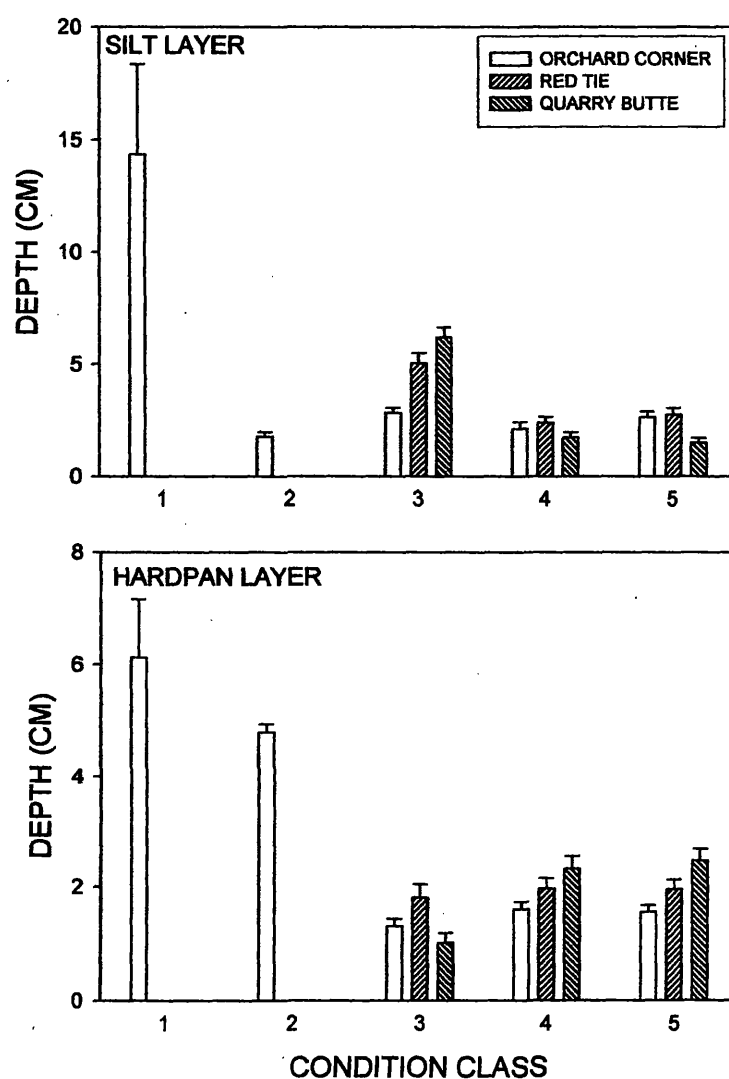


Figure 1. Mean depth of the silt layer and the hardpan layer in slick spots representing three geographic areas and five condition classes. Error bars represent standard error of the mean.

Slick spots that superficially resembled high-condition slick spots but that were not known to support LEPA (Class 3) had relatively thin hardpan layers, slightly thinner than occupied slick spots, with a mean of 1.4 cm. Their silt layers, on the other hand, were thicker, especially in the Red Tie and Quarry Butte microsites, where silt thickness averaged greater than 5 cm. At Orchard Corner, Class 3 slick spots had silt layers comparable in thickness to Class 4 and 5 slick spots.

Class 2 slick spots at Orchard Corner, where LEPA once was present but which had been seriously disturbed and degraded, had silt layers comparable in thickness to the silt layers found in slick spots belonging to Classes 4 and 5, but the hardpan layer in these degraded slick spots was much thicker, averaging 4.5 cm. The process by which the hardpan could increase in thickness as a consequence of disturbance is not known. In Class 1 slick spots at Orchard Corner, where even the surface slick spot appearance of formerly occupied microsites was obliterated, the hardpan layer was even thicker, averaging 6 cm. The silt layer on these obliterated microsites was also much thicker than silt layers on still-evident microsites, averaging 14 cm. This indicates that the microsites were probably obliterated by silt deposition from adjacent matrix soils. The ontogeny of the thick hardpan layer is harder to explain, but could have a major effect on slick spot hydrology and on LEPA seedling establishment.

Matrix soil samples adjacent to slick spots of all classes and in all three geographic areas usually had no hardpan layer. The hardpan layer was missing from 70 to 95% of matrix soil samples (Figure 2). Sporadically occurring samples had sometimes very thick hardpan layers, resulting in means well above zero and large standard errors (Figure 3). These hardpan layers could represent remnant slick spots that have been obliterated at some time in the past. All the matrix soil samples had relatively thick silt layers, with an average thickness of 12 cm.

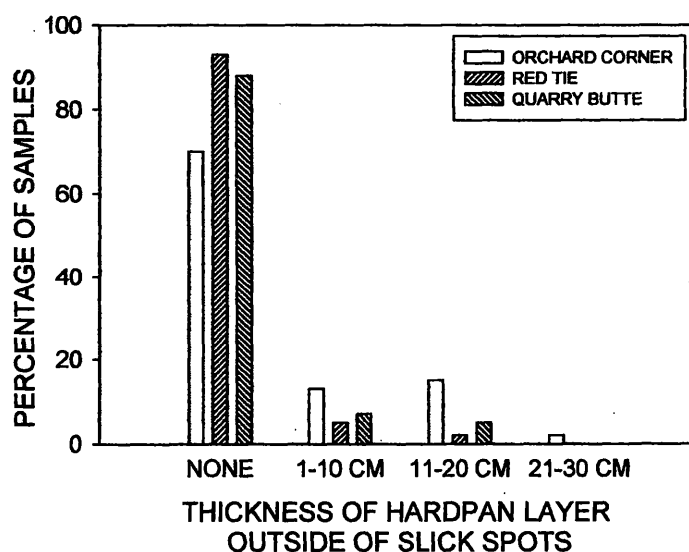


Figure 2. Percentage of matrix soil samples that had no hardpan, a hardpan 1-10 cm thick, a hardpan 11-20 cm thick, and a hardpan 21-30 cm thick, in each of three geographic sample areas.

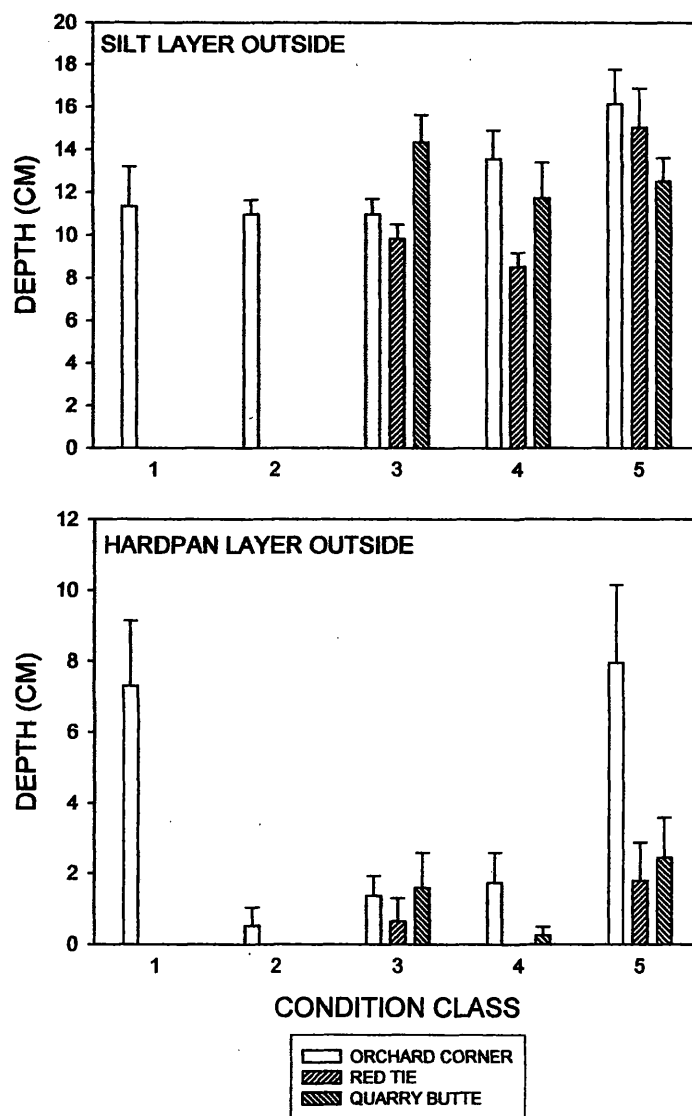


Figure 3. Thickness of the silt and hardpan layers in matrix soils outside of sampled slick spots belonging to five condition classes and located in three geographic areas. Error bars represent standard error of the mean.

For Orchard Corner, there was a trend for thinner matrix soil silt layers adjacent to the lower-condition microsites. This could be due to the fact that the silt that is added to slick spots as they are obliterated must come from the surrounding soil, tending to make the matrix silt layer thinner and to equalize the difference in elevation that caused the slick spots to be sites that collected water in the first place. The support for this idea is not strong in the present data set, however. We really need to sample Class 1 and 2 microsites in the other two geographic sample areas to determine whether this trend is supported.

The most puzzling result of the soil sampling is that the hardpan becomes thicker in



response to disturbance and to silting over. This seems to happen quickly, in a matter of decades if not years, which seems to controvert the idea that such soil layers take a long time to form. Increased hardpan thickness could present obstacles to LEPA emergence and establishment over and above the problems created by a too-thick silt layer.

### Study Question Two: LEPA Seed Banks

We were able to detect living LEPA seeds in soil seed bank samples using the techniques described above, but we did not detect patterns that were easily interpretable in terms of our a priori hypotheses. Part of this difficulty was due to the fact that at the slick spot level our sampling intensity was apparently not adequate even to detect the presence of LEPA seeds in the seed bank in all cases, let alone get a quantitative estimate of seed density. Yet in spite of this low sampling intensity, we did detect strong patterns in the data. The first pattern was a major difference in detectable seeds per sample as a function of geographic sample area. If we look only at microsites belonging to condition classes 3, 4, and 5, which were included for all three geographic sample areas, we find more than a 20-fold difference in mean seed density. The Orchard Corner Class 3, 4, and 5 microsites yielded a total of 117 live seeds, while the Red Tie microsites yielded a total of 31 seeds and the Quarry Butte microsites yielded a total of only 5 seeds. It is hard to see how these major differences could be an artifact of sampling, when 150 sample points were included for each geographic area. But even at Orchard Corner, most seed bank samples yielded no seeds, and a few samples with large numbers of seeds yielded a disproportionate fraction of the total (Figure 4). It may be that with more samples this difference

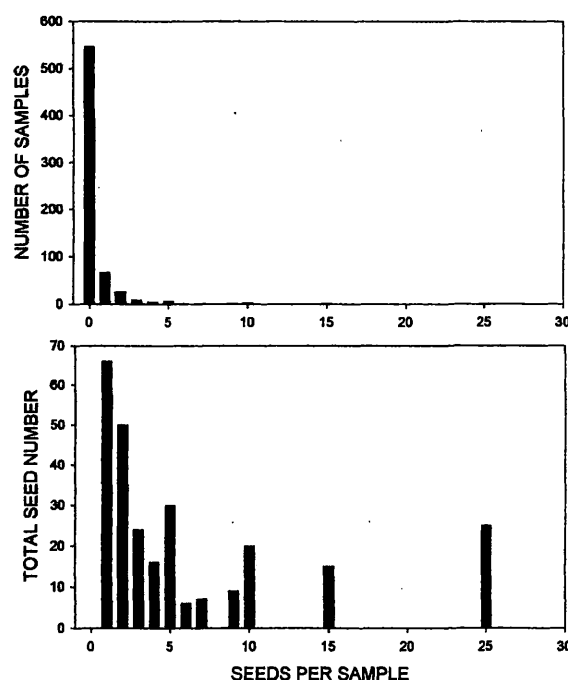


Figure 4. Frequency distribution of Orchard Corner samples with different numbers of seeds, and distribution of total numbers of seeds among samples with different seed numbers.

between geographic areas would be less pronounced, but it is unlikely that it would disappear. These data are difficult to analyze statistically because of their strongly skewed distributions and the large number of zeros in the data set.

Another rather unintuitive result for Condition Class 3, 4, and 5 microsites was the relative lack of correlation between class and seed density.

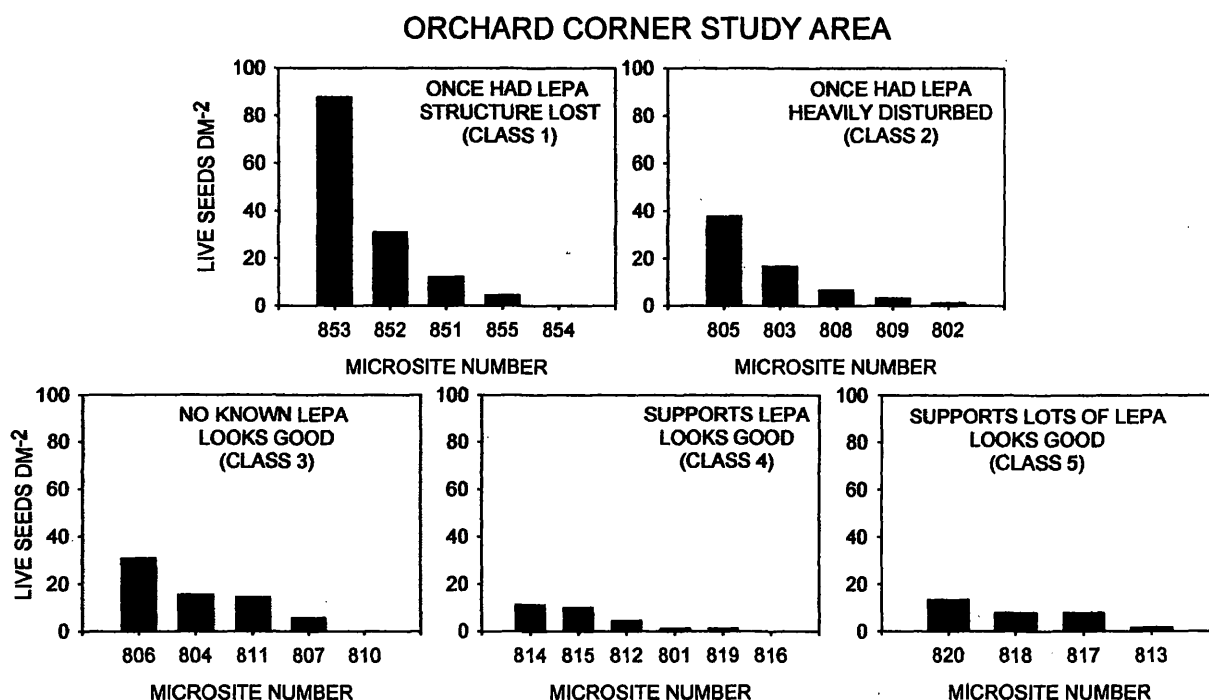


Figure 4. Live seeds per square decimeter for twenty-five slick spots belonging to five condition classes in the Orchard Corner geographic sampling area. Within each condition class, slick spots are ranked by seed density.

For example, at Orchard Corner, the mean seed density for microsites with no known history of LEPA occupation (13.3 seeds per square dm) was actually higher than the mean seed densities for good (class 4) and excellent (class 5) sites (3.6 and 7.6 seeds per square dm, respectively). In both Class 4 and Class 5, there were slickspots where very few or no seeds were found, in spite of the existence of an extant LEPA population. This is clearly a problem of sampling intensity. The Class 3 slick spots that had seeds also had soil structure conducive to LEPA success (thin silt and thin hardpan), so it is possible that LEPA had just been overlooked on these microsites in years past.

In the Red Tie area, few seeds (mean of 0.9 per square dm) were found in Class-3 slick spots, which had thicker silt layers than Class 4 and 5 slick spots (Figure 1, 5). Only one of five Class 3 slick spots yielded live seeds. Class 4 slicks spots yielded a mean of 2.8 seeds per square dm while Class 5 slick spots yielded a mean of 3.4. While these trends are in the predicted direction, the small numbers coupled with the fact that four of nine Class 4 and 5 microsites yielded no seeds makes any firm conclusion impossible.

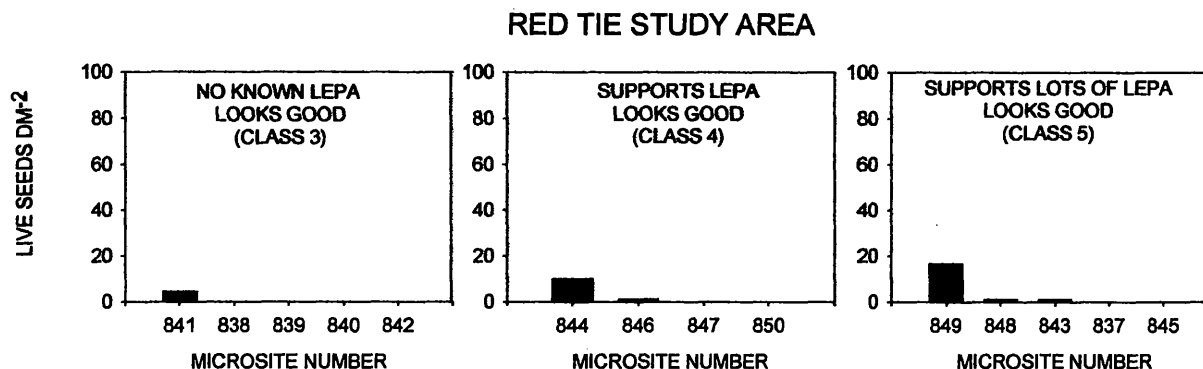


Figure 5. Live seeds per square decimeter for fifteen slick spots belonging to three condition classes in the Red Tie geographic sampling area. Within each condition class, slick spots are ranked by seed density.

Results are even more tentative for the Quarry Butte microsites. Class 3 microsites had no live seeds, but this was almost equally true for Class 4 and 5 microsites, which had a soil structure that was apparently conducive to LEPA occupancy and actually had extant populations at the time of sampling. There is some chance that the low seed number encountered could be related in some way to processing error, but all the samples were processed by the same people using the same technique, so this is not likely. It does seem strange that the seed density is directly related to the order in which the samples were processed, but there is no reason to believe that this could have affected the results.

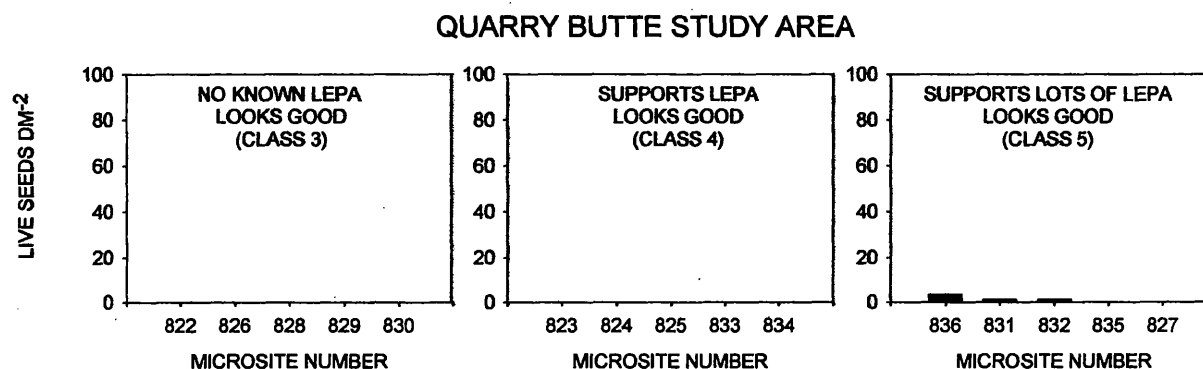


Figure 6. Live seeds per square decimeter for fifteen slick spots belonging to three condition classes in the Quarry Butte geographic sampling area. Within each condition class, slick spots are ranked by seed density.

The seed density results from the Orchard Corner Class 1 and 2 microsites were even more counterintuitive (Figure 4). The mean seed density for Class 1 slick spots, where silting in had obliterated most slick spot features, was the highest encountered, 27 seeds per square dm, while the heavily disturbed Class 2 slick spots had a higher mean seed density (13 seeds per

square decimeter) than Class 4 and 5 slick spots. The presence of relatively large numbers of seeds in Class 1 and 2 slick spots, that no longer support actively growing LEPA, was not expected. In the Class 1 slick spots, most of the seeds were in the hardpan layer, buried under an average of 14 cm of silt (Figure 7). These seeds, as well as the seeds in the red clay layer below, are not likely to be part of the active seed bank. The fact that they are still viable and ungerminated in relatively large numbers suggests that seed dormancy loss and germination are suppressed when the seeds are deeply buried. It may be that results of our retrieval experiment, based on seeds buried approximately 1 cm deep, underestimated the length of time that seeds can persist at greater depth, even though it provided a good estimate of seed longevity in the zone from which seedling emergence can take place successfully. It also means that the presence of viable LEPA seeds in a slick spot may not be a good indicator of population viability.

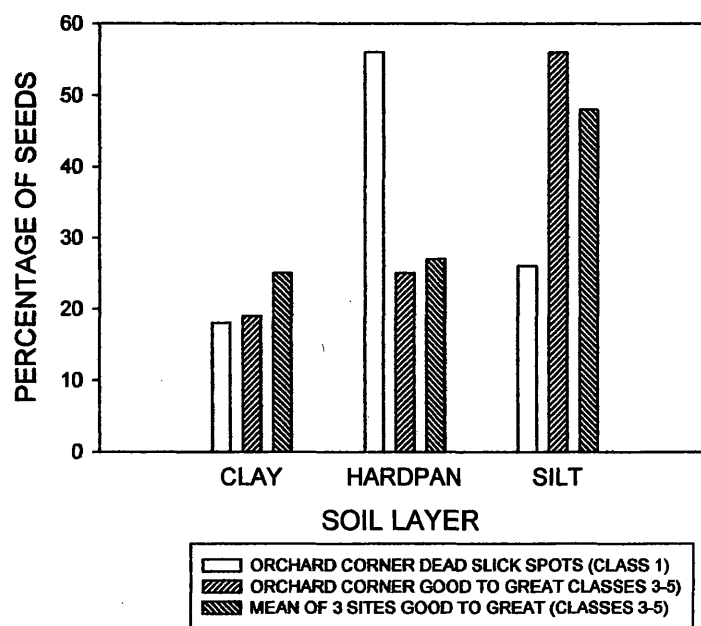


Figure 7. Percentage of seeds retrieved from the red clay, hardpan and silt layers for Orchard Corner dead slick spots (Class 1), Orchard Corner Class 3, 4, and 5 slick spots, and the mean of Class 3, 4, and 5 slick spots for all three geographic sample areas.

Most of the seeds in high condition slick spots were in the silt layer, which averaged only 2 cm in thickness on these microsites. Even those in the hardpan layer were often still close enough to the surface to be able to emerge successfully (see section below on emergence from depth). Seeds in Class 2 slick spots were also mostly located in the relatively thin silt layer (71%) and were potentially part of the active seed bank. Establishment failure on these slick spots could be related to the much thicker hardpan layer (Figure 1). The presence of viable seeds in a relatively thin silt layer would lead to the apparently erroneous conclusion that these microsites still support LEPA populations. This means that seed bank surveying would not be a reliable way to assess LEPA population status for such microsites.

In Class 3, 4, and 5 (good to great) slick spots, there was no overall tendency for seeds to be located in any particular part of the slick spot. Seeds occurred in approximately equal densities in the center, middle, and edge of a microsite. In the Orchard Corner Class 1 slick spots, by contrast, seed densities were approximately twice as high at the edge. The Orchard Corner Class 2 slick spots had higher seed densities on average in the middle section.

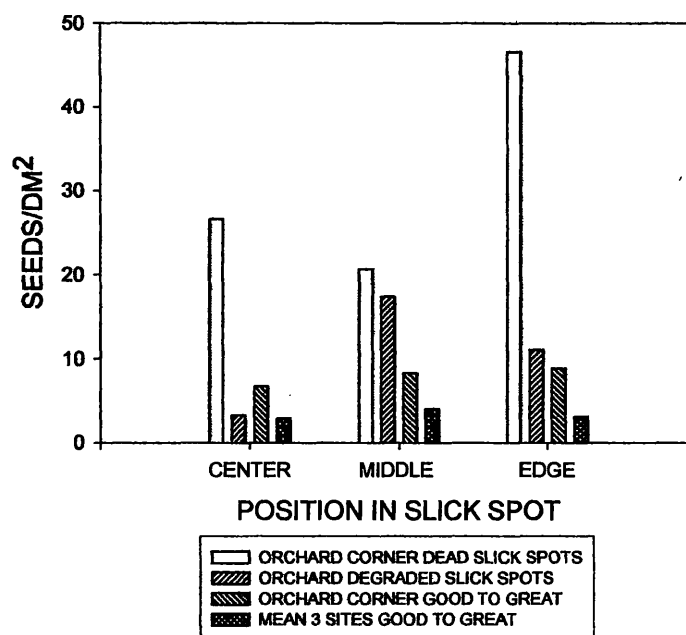


Figure 8. Mean seed densities in different microsite positions for Orchard Corner Class 1 microsites, for Orchard Corner Class 2 microsites, for the mean of Orchard corner Class 3, 4, and 5 microsites, and for the mean of Class 3, 4, and 5 microsites over three sampling locations.

Conclusions on LEPA seed density and distribution in different condition classes of microsites and in different sampling locations based on the 2004 data must be considered tentative. In order to really quantify seed density, we probably need much larger sample sizes. We also need to narrow down the thickness of the layer from which a seed bank sample is taken. Without doing this, we cannot tell whether seeds are in the active seed bank from which emergence and establishment is possible, or whether they are entombed alive at depths from which they can never emerge. This problem is not so great on high condition slick spots, which generally have silt and hardpan layers only a few centimeters thick. But to interpret seed sample data from degraded slick spots, we need to know, for example, exactly where in the 14-cm-thick silt layer the seeds were located. For purposes of quantifying the active seed bank, the best approach would probably be to determine the maximum depth from which LEPA seeds can emerge, and to consider any seeds found more deeply buried to be no longer part of the active seed bank. Once that depth is known, it would be necessary only to sample the surface increment that contained the active seed bank.



### Study Question Three: LEPA Seedling Emergence

There was a clear trend in our greenhouse emergence trials for decreasing emergence with increasing depth over the range from 2 to 30 mm (Figure 9). Emergence varied from a mean of 54% at the shallowest planting depth to a mean of 5% at the 30 mm depth. The fact that the 10/20C experiment was run separately from the 5/15 -20/30 experiment and that it did not include the 30 mm depth treatment makes the data a little difficult to interpret.

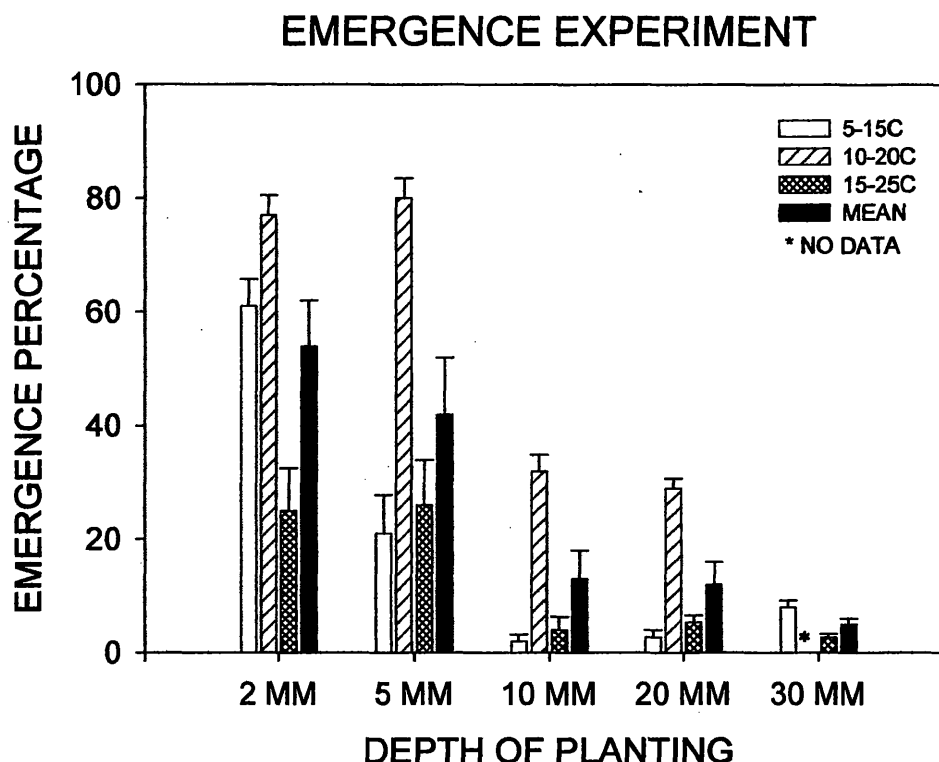


Figure 9. Emergence percentage after planting seeds in slick spot soil under water non-limiting conditions at three temperatures and five depths.

Seedling emergence was higher overall at 10/20C than at the other two temperatures, as high as 80% at the two shallowest planting depths. Averaged over the depth treatments in common, emergence was over twice as high at this intermediate temperature. This could have been an artifact of the seed poking technique, however. It may be that the technician who poked the seeds for the first experiment damaged the seeds less than the person who poked the seeds for the second experiment. For this reason the among-temperature comparison must be interpreted with caution. We certainly saw no indication of improved emergence from greater depth at the lowest temperature.

Mean seedling emergence dropped slightly from 2mm (54%) to 5 mm (42%). There was a sharp decline when seeds were planted at 10 mm (13%) but little further decline at 20 mm (12%). Seedling emergence was very low from the 30 mm planting depth, but the lack of data for the

10/20 treatment may have skewed this figure downward somewhat. It is remarkable that emergence was possible at all from this depth for such a small seed. It appears that LEPA is adapted for emergence from greater depths than would be predicted from its seed size. This would make successful emergence in a rapidly drying soil more likely, and would also compensate for the negative effects of burial resulting from the seeds sinking down into the mud when the slick spots are wet in winter.

We are in the process of repeating the emergence experiments with a completely balanced design to confirm the results described above, with the inclusion of one more depth treatment, 40 mm. A provisional recommendation for taking seed bank samples in the field is that seeds that are located >40 mm (4 cm) deep can be considered to be lost from the active seed bank because of too-deep burial. For quantifying only the active seed bank, samples to a depth of 4 cm should be adequate.

#### **Study Question Four: Life History Expression**

The experiment to determine whether life history expression has a genetic basis was initiated on January 18, 2005. Emergence was generally poor in this experiment, much lower than that obtained in our emergence trials in native soil. We attempted to plant at 5 mm depth, but depth of planting was hard to control in the fluffy potting mix we used, and many seeds may have been planted too deeply. Also, a third person poked the seeds for this experiment, and she allowed the seeds to dry after poking before re-imbibing them for chilling. This could have affected seed vigor. Another possibility is that the seeds were subject to disease in our high organic matter potting mix, even though it had been steam-pasteurized. We often see low emergence from seeds that have to be artificially injured to induce germination, for example, hard-seeded legumes and globemallows.

From a total of 392 planted seeds, we obtained a total of 71 plants, about 18% of planted seeds. Of these 71 plants, 11 died soon after emergence. Once established, the remaining 60 plants grew quickly and well in the tube containers, achieving a mean rosette diameter of 70 mm. One of our goals was to determine how easily seeds could be grown to produce tissue for genetic analysis; these plants yielded ample tissue for such an analysis. The plants were grown in a greenhouse under day neutral conditions (12:12 h light:dark) using supplemental light through March 21, at which time natural day length increased beyond the 12 hour light part of the cycle. As of April 19, most of the plants have still not flowered, presumably because day length has not increased sufficiently to trigger the photoperiodic flowering response. All of the plants are well beyond the field threshold size for flowering. To date, 3 of 31 biennials and 3 of 29 annuals have flowered. There is thus no evidence so far for a genetic basis for the flowering response, as annuals and biennials are behaving similarly. We expect the rest of the greenhouse-grown plants to initiate flowering more or less synchronously with plants under field conditions, some time in May. This experiment will be scored and terminated on June 21. Day length begins to shorten after this day, and any photoperiod response should long since have been triggered. We plan to repeat this experiment under growth chamber conditions where both day length and temperature can be controlled and manipulated.

## Plans for Further Research

The studies reported upon here have answered several important questions about LEPA ecology and life history, but many issues remain unresolved and many questions unanswered. First, we need to find out whether our soil and seed bank results for Orchard Corner Class 1 and 2 microsites are typical of LEPA habitat as a whole. The thickening of the hardpan that seems to be associated with disturbance was an unexpected result that requires further verification and explanation. This was associated with emergence failure of an apparently active seed bank in Class 2 microsites and with entombment beneath a thick silt layer and apparent prolonged dormancy in Class 3 microsites. These results need to be confirmed. The most elegant way to do this would be to carry out the sampling for Red Tie and Quarry Butte in the same way that it was done for Orchard Corner. This would result in 5 slick spots x 2 geographic areas x 10 samples per slick spot x 3 layers = 300 additional samples.

Second, we need to do some intensive sampling in Class 4 and 5 slick spots to find out how big the sample size needs to be to get a repeatable estimate of seed density. We need to take these additional samples from slick spots already sampled so that we can make use of existing data. We could start by re-sampling three previously sampled Class 5 slick spots in each sampling area, including those that yielded no seeds and those that yielded the highest seed density. Sampling the silt, hardpan and first 2 cm of the clay layer as we did before should work for these slick spots, which have relatively thin silt and hardpan layers. We could measure the thickness of these layers as before. We could start with 20 additional samples from each of these nine slick spots for a total of  $20 \times 9 \times 3$  (layers) = 540 samples. If we are still getting widely oscillating density estimates going from 20 to 30 samples, we will go back out and get another 20 samples from each of these slick spots. Once we know the sample size necessary to adequately sample a slick spot seed bank, we could go back to all the Class 2, 3, 4, and 5 slick spots and resample.

Third, we need to repeat the emergence experiment to verify the depth from which LEPA can successfully emerge; this experiment is already underway. We also need to finish up our preliminary study on LEPA life history expression and, depending on how it turns out, design and execute further studies to learn how to consistently trigger flowering in the greenhouse. Fifth, we need to learn how to cross pollinate greenhouse-grown plants and get them to produce quantities of viable seeds for research on restoration of imperiled LEPA populations and reintroduction of those that have been either extirpated or that have become locally extinct through natural processes. Learning how to restore LEPA populations will mean incorporating the genetic information being generated by the team at Boise State with the technology we develop for producing seeds in the greenhouse. It will also involve field evaluation of slick spots to determine whether they are suitable habitat. At this point, the data seem to suggest that a hardpan that averages more than 3 cm in thickness and/or a silt layer that averages more than 4 cm in thickness would render a slick spot uninhabitable for LEPA. More work to verify this result would also be useful. It would not need to include the seed bank part--we could just go to highly disturbed or dead slick spots and measure these layers as we did before. Quantifying the seed bank itself in these disturbed slick spots has become less of a priority, given that the presence of seeds in a suitable location for emergence does not equal a successful population.